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STRUCTURE OF THE *CULEX* EGG AND EGG-RAFT IN RELATION TO FUNCTION (DIPTERA)

By Bt.-Col. Sir S. Rickard Christophers, C.I.E., O.B.E., I.M.S. (retd.).

Manuscript received 16 September, 1944.

(Read 1 November, 1944.)

With 4 Plates.

The egg-raft of *Culex pipiens* is a common object to be seen floating on the surface of water butts and similar collections of water in this country. Many other species of *Culex* lay their eggs in the form of rafts as do also certain species of the unrelated genus *Theobaldia*.

These egg-rafts float so lightly on the water that they are blown about by the least movement of air, yet withstand any attempt to upset or sink them. Should the raft by some violent action be submerged it rapidly regains the surface and, if undamaged, not only floats again but does so correctly right side up as it was before submergence. This property of the egg-raft is dependent on certain structural features of the egg and the arrangement of the eggs in the raft. It will be convenient to describe first the structural features of the egg itself and to follow this with a description of the arrangement of the eggs in the egg-raft. I shall then discuss correlation of the structures described with function, and note such specific differences as have been observed in species so far studied.

The Egg.

The egg of *Culex pipiens* and its near British relative *Culex molestus* measures in length somewhat less than a millimetre (0·73 mm. in length by 0·15 mm. in greatest breadth [1]). It is elongate oval in shape, thicker and more rounded anteriorly and thinner and more pointed at its posterior end. It is slightly curved in its posterior third with the pointed posterior end directed somewhat dorsally (Plate 2, fig. 1). Conspicuously perched on the anterior pole of the egg is a peculiar transparent cup-shaped structure, the *micropilar apparatus* or *micropilar cup*. This structure would appear to be confined to species of the raft-laying genus *Culex* and its near relatives. When the micropilar apparatus becomes detached, which readily happens from mechanical damage, there is displayed a curious projection, the *egg-spike*. Around the egg-spike on the anterior extremity of the egg is an elaborate radial arrangement of ornamental thickenings, the *anterior polar specialised area*. At the posterior pole is another much smaller specialised area, the *posterior polar specialised area*. Apart from these special structures at the poles the egg surface is uniformly smooth throughout.

The exochorion.

The egg is covered externally by a very delicate semi-transparent membrane, the *exochorion*, which is readily injured and detached. It is formed from the

[1] These measurements are, however, subject to slight increase as hatching of the larva approaches (see under egg-cap and hatching).

layer of follicular cells which surround the ovum and nurse cells (Vishna Nath, 1924) and should, strictly speaking, be the true chorion. It is convenient, however, to distinguish it from an inner layer, the endochorion, which is the product of these cells. The exochorion appears to be of a waxy nature as it shrinks to an almost shapeless mass when eggs are treated with ether. Under a low power it is characteristically finely stippled. Under the oil immersion this stippling is seen to be due to the presence of closely set small cylindrico-conical projections. These projections are shaped rather like the modern rifle bullet and are set in a delicate membrane showing a double contour for all the world like metal studs in leather and when dissected free they show a rim at the level where they pass through the inner layer of the membrane (Plate 3, fig. 3). They measure only about 4 μ in length by 1·5 μ in breadth and are spaced rather regularly at a distance from one another about equal to their thickness. This highly characteristic structure of the exochorion has been found present in all species of *Culex* of which the eggs have been examined and in *Theobaldia annulata*.

At the anterior pole of the egg the small regular projections, which cover uniformly the whole egg surface, become changed to form the radially arranged plates bearing tubercles that together constitute the anterior polar specialised area. At the posterior pole the projections again become enlarged and specialised to give rise to the posterior polar specialised area.

Around the egg-spike the exochorion is reflected as a thin membrane into the base of the micropilar cup.

The surface of the exochorion, except at the posterior polar specialised area, is highly hydrophobic and non-wettable. It gives to the general surface of the egg in an undamaged condition these characters in a high degree. When, however, from mechanical injury any portion of the exochorion becomes detached, areas so robbed of this layer are no longer hydrophobic and if damage is extensive such an egg becomes wetted at the damaged spot and at once liable to be involved in the surface film.

The endochorion.

Beneath the exochorion is a thick opaque elastic layer, the *endochorion*. This is a secretion laid down by the follicular cells (Vishna Nath, 1924). It is a hard, rigid, tough and elastic membrane, some 3–4 μ in thickness, which gives to the egg its shape and consistence and may in fact be regarded as the egg-shell. It is of nearly uniform character and thickness over the whole egg, except at the anterior pole where it becomes somewhat thicker and is continued into the dense egg-spike. It is this layer which at hatching is torn to allow escape of the larva, forming in the process the egg-cap.

There is some evidence that lying between the exo- and endochorion is an oily or lipoid layer. At least it can be said that the two membranes are quite unconnected and that the exochorion when stripped off leaves the endochorion with a shiny smooth glistening surface, while there appear to be liberated some globules of an oily nature. In support of the view that there is an oily or lipoid layer between the two coats of the chorion is an interesting phenomenon that is seen when an egg-raft is placed in ether. The exochorion shrinks and ruptures, allowing the contained smooth endochorion-covered eggs to slide out and fall into the fluid in a manner suggestive of heavy ammunition shells upset from a dump.

The structure of the egg within the endochorion does not concern us in the present connection.

The micropilar apparatus.

Of all the external structures of the egg the most striking is the micropilar apparatus. It is formed from that part of the follicular epithelium which surrounds the nurse cells (Vishna Nath, 1924). In the undischarged egg it forms a closed sac traversed in the median line by a delicate membranous channel, the *micropilar funnel.* In it at this stage also lie the remains of the nurse cells. Even immediately after oviposition, however, its apical portion has disappeared so that the intact bladder has become an open cup. The micropilar funnel is no longer to be made out, except perhaps its lower end where it abuts upon and surrounds the egg-spike. In a newly laid egg, however, there is usually a gelatinous substance still present in the cup which is evidently derived from the dissolution of the nurse cells.

As seen after oviposition, the micropilar apparatus forms a flat-bottomed cup. The floor of the cup is thickened with irregular network-like thickenings and is relatively rigid. The walls, however, are formed of a delicate flexible membrane with radial parallel thickenings forming ridges on its inner aspect. These appear as striations arranged in groups of 5–6 and giving an effect as if the sides of the cup were formed of separate petals. These " petals " number usually about 30 and correspond in position with the 30 or so radial thickenings of the anterior polar specialised area. In sections the striations are seen to be thickened ridges lying on the inner aspect of the cup walls with deep grooves between them. A somewhat systematised view of these ridges is given in Plate 3, fig. 6. The inner surface of the cup is highly wettable and hydrophilic.

The walls of the cup are very flexible and expandable, and the shape and appearance of the cup vary under different conditions. When in contact with the surface film the delicate walls of the cup are expanded to become almost flat and disc-like. When dragged from contact with the film the cup carries away with it a large drop of fluid on to which the walls are collapsed and incorporated (Plate 3, fig. 7, b). The cup now appears as a glistening transparent globe. As the contained fluid dries the apparatus assumes the appearance of a cup, which is the usual state in which it is seen on examination. With still further drying it becomes flattened out on to the anterior pole of the egg.

Because of the strongly hydrophilic micropilar cup and the strongly hydrophobic exochorion covering the rest of the egg, an egg, if uninjured, can be picked up by touching the micropilar cup with a wet needle, the egg then standing out at right angles to the needle. Or a single egg by the same mechanism can float upright on the surface of the water like a molecule in a mono-molecular layer. If the exochorion, however, be damaged conflicting hydrophilic areas are formed and the egg adopts a position in accordance with the new conditions.

The anterior polar specialised area.

The modified exochorion forming the anterior polar specialised area appears as a series of about 30 narrow thickened plates radially arranged around the egg-spike. Each plate carries a double row of tubercles, representing enlarged exochorion projections. The whole has a highly ornamental effect (Plate 2, fig. 4). Each of the plates corresponds in position to a petal of the micropilar cup and if one remembers that in its natural position the weight of the egg, with possibly some pressure from the weight of the raft as a whole, rests upon the micropilar cup it seems not improbable that this rather elaborate ornamenta-

tion is designed to give protection to the delicate contents of the developing egg in this situation.

The posterior polar specialised area.

At the posterior pole of the egg and slightly on the dorsal aspect is a specialised area of the exochorion. This consists of a circular area 0·04 mm. in diameter over the outer portions of which the exochorionic projections are larger than those on the general surface of the egg, and, in addition, are armed with multiple spikes giving them the appearance of burrs or the prickle cells in the human epidermis. These prickle-bearing projections increase in size inwards until they end in a row of specially large spiky projections surrounding a small central circular area of delicate exochorion devoid of any thickenings. This area of smooth membrane lies just over that part of the egg containing the embryonic tissue where development of the embryo is initiated. It would appear to have an important function of some kind. This and the function of the prickle projections will be discussed later.

The egg-spike.

The only remaining structure that we need call attention to is the egg-spike. In the more or less mature egg this appears as a thickening of the endochorion projecting into the base of the micropilar cup. In the newly laid egg, however, it can be seen as a distinct structure set in the endochorion like a watch-glass on the face of a watch. At this stage it contains a spongy interior but no traceable channel. Since it lies at the inner end of the micropilar funnel it must almost certainly lie in the path of the fertilising spermatozoon and so be traversable at some stage of its existence. Very probably it acts as a sort of post-fertilisation plug. In addition it almost certainly gives important support to the micropilar cup.

The Egg-raft.

The egg-raft in those species of *Culex* that have been examined, viz., *C. pipiens*, *C. molestus* and *C. fatigans*, usually consists of from 100 or less to about 400 eggs, depending on the species of mosquito laying it. Viewed from above or below, the raft is usually broadly oval in outline, most commonly blunter at one end than at the other, giving it a somewhat pear-shaped outline. The more pointed end is probably that at which the last eggs are laid, though this has not been confirmed by actual observation.

The raft is boat-shaped in so far as the lower surface is somewhat the larger and convex, whilst the upper surface is smaller and concave. Actually it is more like some form of native sampan than either a boat or a raft (Plate 1).

The eggs lie in longitudinal rows, those in each successive row alternating in position so that the eggs of one row lie opposite the line of meeting of two eggs in the succeeding row. This gives the raft, looked at from above or below, a very regular hexagonal honey-combed appearance. The two longest rows lie on either side of the mid-longitudinal bisecting line of the raft and succeeding rows lying outside these become shorter, with fewer and fewer eggs, until the outermost rows on either side may consist of some four or five eggs only. The number of such rows on either side of the median longitudinal line and the number of eggs in the rows vary according to the species.

In each row the eggs are oriented so that their somewhat concave dorsal aspect lies directed outwards. This arrangement is very regular throughout

most of the raft, but is apt to be a little modified towards the two ends of the raft and notably so towards the more pointed end. When in the course of development the dark eye spots of the contained larvae become visible in the eggs these are seen to be very regularly placed laterally, *i.e.*, facing towards either end of the raft. Since along the middle bisecting line of the raft the two median rows face in opposite directions, it follows that there is a slightly wider space between the posterior poles of the eggs in these two lines than between other rows. This may be so marked in large well-formed rafts, like those of *C. pipiens*, that a distinct median longitudinal sulcus is present in this situation.

Since the eggs are relatively rigid and retain their cylindrical form in the raft, a small space is left at each angle of what would be the hexagon that would be formed if the eggs were compressed into this shape, *i.e.*, compacted as are the cells in a honeycomb. Such spaces are quite conspicuous when a raft is viewed by transmitted light under the microscope. They traverse the egg-raft from its lower to its upper surface, making some hundreds of perforations in its structure. It is entirely due to the fact that the exochorion is strongly hydrophobic and non-wettable that water does not enter these spaces. On the contrary they form air-locks and remain permanently full of air, adding greatly to the buoyancy of the raft. This is very strikingly shown if a raft be placed in some fluid which allows of wetting of the egg surface. Then since the eggs are heavier than water the raft sinks at once, the air contents of the spaces being liberated as bubbles. It is not therefore merely because it is boat-shaped that the raft floats, though given buoyancy by its air spaces the convexity of the lower surface may play some rôle.

The cohering mechanism.

It is a natural assumption that the eggs are held together to form the raft by some cementing substance. Careful examination has, however, failed to demonstrate any such substance. Single eggs or whole rows of eggs are readily detached by the least touch of a needle. Nor, if care be taken, is the exochorion necessarily torn or visibly damaged after such a procedure. Further, if after being so separated eggs are examined, even after staining of the exochorion, no mark indicating where the eggs have been in contact has been made out. The impression after such manipulation is that if there be any cementing substance it is of the nature of a thin oily coating. But if an egg-raft be treated with ether the eggs do not separate and fall apart; rather the whole exochorion component of the raft shrinks and expels the eggs entirely free from exochorion as already described. A further point in this connection is that when the larvae hatch and leave the raft, then the eggs, now reduced to shells, do very largely fall apart, retaining their exochorial covering. It seems legitimate, in fact, to ask whether cohesion of the eggs is not brought about by an interlocking of the small conical projections already described as so characteristic of the exochorion, *i.e.*, that the eggs adhere in much the same way as would two rubber coverslip mats if pressed together face to face. Some attempt to ascertain from thin sections whether this might be so has been made. It is only possible, however, to say that appearances are compatible with such a possibility.

The apical droplet.

A conspicuous feature of an egg-raft which does not seem to have been previously described is that if examined in its natural condition floating on water, or suitably kept moist, there will be seen poised on the pointed posterior

end of each egg a small droplet. Provided the egg-raft has not been allowed to remain any length of time away from water, the presence of such droplets is invariable. They are of similar size and appearance on every egg, and this, combined with their geometrically regular arrangement, further helps to give to an egg-raft viewed under suitable illumination with a low-power binocular microscope its generally beautiful effect. The droplets (Plate 3, fig. 8) are globular, measure somewhat under 0·05 mm. and are in contact at one point with the posterior polar specialised area covering the minute central papilla-free portion of exochorion. These droplets appear shortly after oviposition and if removed they reform. They are presumably condensed from the atmosphere by the prickly processes already described. As well as being observed in all species of *Culex* examined, they are also present on the egg-raft of *Theobaldia annulata*. It seems very probable that the droplet has some function either protective or concerned with passage of water into the egg.

The egg-cap and hatching.

In the later stages of development the egg increases in size from a length of about 0·73 mm. to 0·8 mm. or slightly more. For some hours before hatching the pigmented eye-spots of the contained embryo can be seen shining through the egg-shell. In the raft these eye-spots lie strictly oriented facing towards the ends of the raft. From this stage also segmentation of the contained embryo can be seen.

When hatching occurs a portion of the endochorion, with its cap of exochorion, including the micropilar cup and related structures, is forced off at the anterior end of the egg forming the *egg-cap*. This egg-cap is not completely separated but remains attached by a narrow unbroken portion of endochorion. The connecting strip is always situated in the middle of the dorsal aspect. Hence when the larvae hatch from the eggs in a raft all the egg-caps open outwards away from the mid-longitudinal line of the raft except towards the ends of the raft where, as previously stated, the eggs themselves may be a little out of this strict arrangement. An egg-raft from which the larvae have hatched viewed from above shows a regular line all round the edge of these turned-out little caps (Plate 4, fig. 1).

The first-instar larva, as is well known, has an egg-breaker on the middle of the dorsal surface of the head. One would expect then that the break or cut made by this would be most complete dorsally. Actually just the opposite is the case : the cut is complete everywhere except at the mid-dorsal line corresponding to the position of the egg-breaker.

When the larvae have hatched the raft soon becomes partially or wholly broken up.

Structure and Function in the Egg and Egg-raft.

The most important external structural feature of the egg in relation to the egg-raft-forming habit would appear to be the hydrophobic character of the exochorion. Next to this comes the existence at the lower (anterior) end of the egg of the strongly hydrophilic micropilar cup. The hydrophobic egg surface would presumably be sufficient to give the egg-raft great buoyancy, but it would not of itself arrange for the perfect orientation of the raft under all conditions short of mechanical injury. In the powerfully hydrophilic micropilar cups the raft, however, is provided with some hundred or more attachment

points to the surface film. These not only anchor the raft and give it the power to re-orient itself even after violent agitation or submergence, but they give it an almost frictionless freedom of translatory movement over the surface film.

It seems probable too that it is the peculiar structural features of the exochorionic papillae that ensure coherence of the eggs in the raft, allowing a certain non-rigidity of attachment and permitting break up of the raft when the larvae are wriggling themselves free, a feature that might be more favourable to escape than a rigidly cemented egg-raft.

The radially arranged plaques formed from modification of the exochorionic processes and accurately placed opposite the " petals " of the micropilar cup would seem to be not merely ornamentation but a mechanism to give support to the cup and possibly protection from the weight of the egg-raft upon important and delicate embryonal structures within this part of the egg.

The spiky burr-like exochorionic processes at the posterior pole are almost certainly the mechanism responsible for the formation of the condensation droplet, whilst the window of thin membranous exochorion devoid of processes at the extreme pole suggests some functional adaptation in the way of provision for utilising the water in the droplet to ensure hydrostatic adjustment during progress of embryonic development, an adjustment which must in some manner take place, as is evidenced by increase in the size of the egg as hatching approaches.

It may be said then that the habit of laying the eggs in the form of a raft in *Culex* is a procedure intimately connected with and dependent on quite a number of details of structure of the egg and egg-raft, among which the peculiar nature of the exochorionic structure and the presence of a hydrophilic micropilar cup are predominant.

Unfortunately very little attention has been given to describing the characters of the egg in mosquitoes other than the Anophelini. The habit of laying the eggs in the form of rafts appears, however, to be usual in mosquitoes of the subgenus *Culex*, a dominant form as regards number of species and abundance in nature. Besides those species already mentioned, *C. acer*, *C. mimeticus*, *C. bitaeniorrhynchus* and others are known to lay their eggs in this form. On the other hand, by far the most usual method in the CULICIDAE is for the eggs to be laid singly as in the dominant group *Aedes*. In *Aedes aegypti* the exochorion is raised into numerous small blebs over the surface of the egg and has quite a different character from that here described in *Culex*. A micropilar cup again seems to be quite restricted to *Culex*. The laying of the eggs in the form of egg-rafts must therefore be regarded as a definite specialisation developed in the *Culex* group and involving much more than merely massing the eggs together. It may therefore be of some phylogenetic importance and hence important in classification.

The only genus other than *Culex* in which raft formation has so far as known to the author been described is in the subgenus *Theobaldia* of the genus *Theobaldia*, it being well known that the British species *Theobaldia annulata* lays its eggs in this manner. The other subgenus in *Theobaldia* as at present constituted, viz., *Allotheobaldia* is recognised as differing in many respects from subgenus *Theobaldia* and does not lay eggs in raft form. From this fact alone some suspicion should exist as to the true affinity of the two subgenera.

Since there is every reason to regard subgenus *Theobaldia* as only very distantly related to *Culex* some interest obviously attaches to the characters of the egg and egg-raft in this group. Owing to the kindness of Mr. G. W. Otter, who has fed some female specimens of *Theobaldia annulata* caught in nature and

obtained egg-rafts for me, I have been able to give at least a preliminary description of the egg in this species. The egg-raft shows an arrangement of the eggs very similar to that in the species of *Culex* here described. There is the same regular arrangement in longitudinal lines, the same orientation with the dorsal surface facing outwards. The exochorion over the general surface of the egg is almost identical with that in *Culex* with minute closely set cylindrico-conical projections. The egg does not possess a micropilar cup. It has, however, an entirely different specialisation at the anterior pole of the egg obviously performing the same function. This consists of a large area occupying most of the anterior polar surface, surrounded and marked off by a thickened white rim or frill, which is entirely devoid of chorionic papillae, and is wettable and powerfully hydrophilic (Plate 3, fig. 9). Actually the same combination of a general hydrophobic egg surface with an anterior polar wettable and hydrophilic area is present as in *Culex*, but the latter character is dependent on a different structural layout.

Specific Differences in the Egg and Egg-raft.

The eggs of those species of *Culex* examined do not differ appreciably in size or structure. The egg-rafts, on the contrary, show quite constant specific characters.

The raft of *C. fatigans* (bred in the laboratory in England) measures 3 to 4 mm. in length by about 1·3 mm. in greatest breadth. It is rather evenly elongate oval in shape. The number of eggs is usually about 150–200 arranged in 4–5 rows on either side of the mid-longitudinal line, the two middle lines being formed of about 25 eggs.

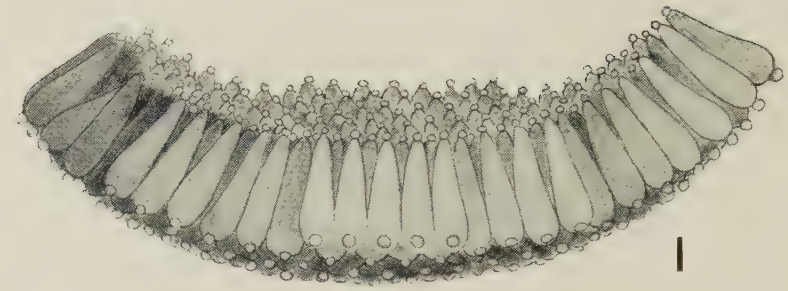
The raft of *C. pipiens* is much larger, measuring as much as 6 mm. in length by 1·8 mm. in greatest breadth. It is rather narrowly oval in outline. The number of eggs is usually about 400, arranged in 6–9 rows on either side of the middle line, the two median rows consisting each of about 40 eggs. The eggs are very regularly arranged and the median longitudinal sulcus is conspicuous.

The raft of *C. molestus* is much smaller than either of the above two species and strikingly so as compared with that of *C. pipiens*. When laid without a blood meal it rarely exceeds 2 mm. in length or consists of more than 80 eggs. Very commonly autogenously laid egg-rafts where rearing conditions have not been very favourable are much smaller, egg-rafts of 50 or even a less number of eggs being laid. Following a blood meal the rafts usually contain from 80 to 100 eggs, very rarely exceeding the latter figure, and may reach about 3 mm. in length. The rafts are very variable in shape, commonly broadly and regularly oval, often triangular in general outline, or sometimes elongate rectangular. A feature of the raft is some irregularity in the arrangement of the eggs, making it more difficult accurately to draw the raft, which rarely if ever shows a conspicuous median groove.[2]

The egg-raft of *C. acer* has not been recently studied, but it can be stated to be of a very characteristic shape, being very long and narrow, somewhat like an Eskimo kayak.

The egg-raft of *Theobaldia annulata* is very similar in general appearance to that of *C. fatigans*, though much larger, due to the larger size of the eggs. It is oval or pear-shaped and consists of about 120 eggs.

[2] Since this paper was read Mr. F. W. Marshall has described the egg-raft of *C. pipiens* and *C. molestus*. His description of the main differences is similar to that here given.



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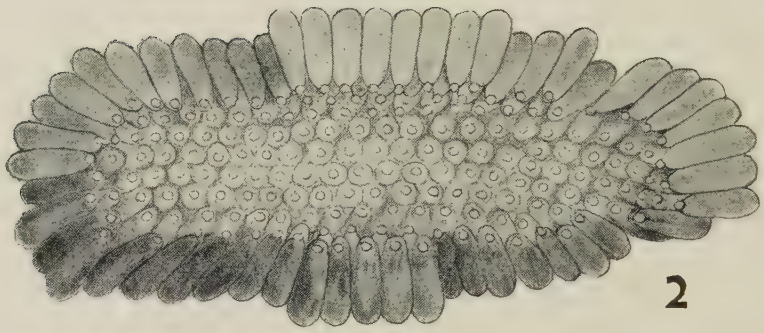

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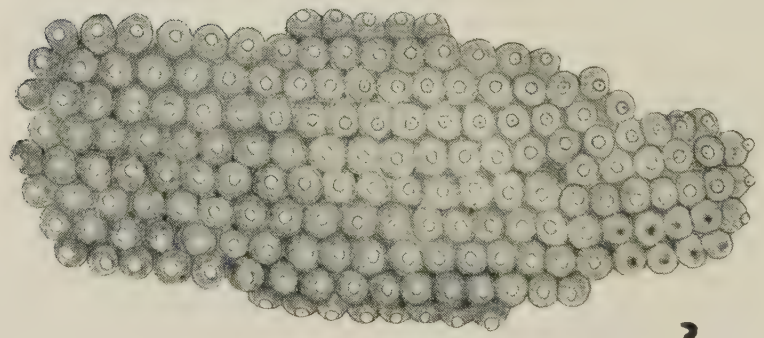
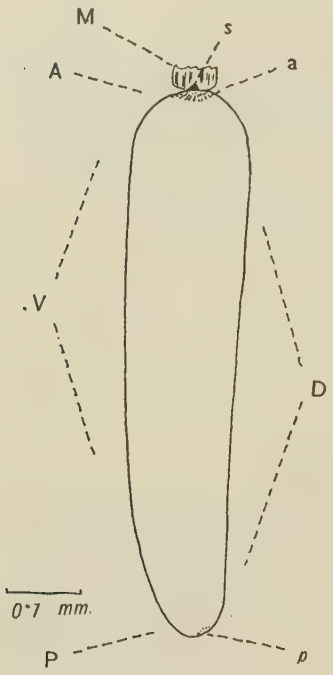


3

1 0 mm.

Egg-raft of *Culex fatigans*.



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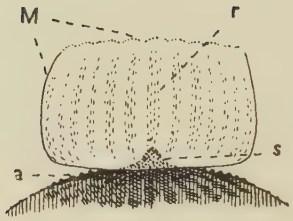

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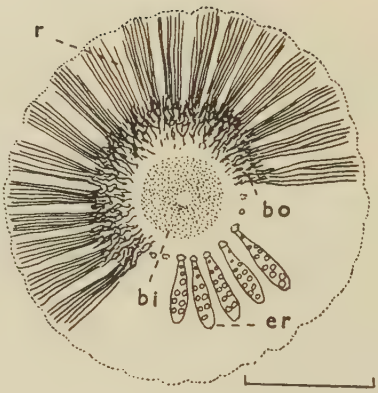

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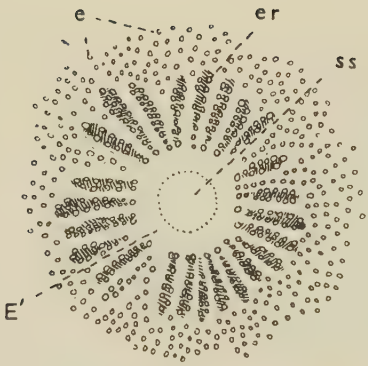

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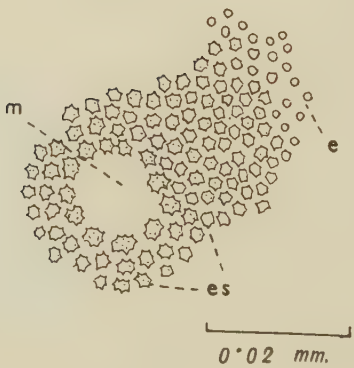

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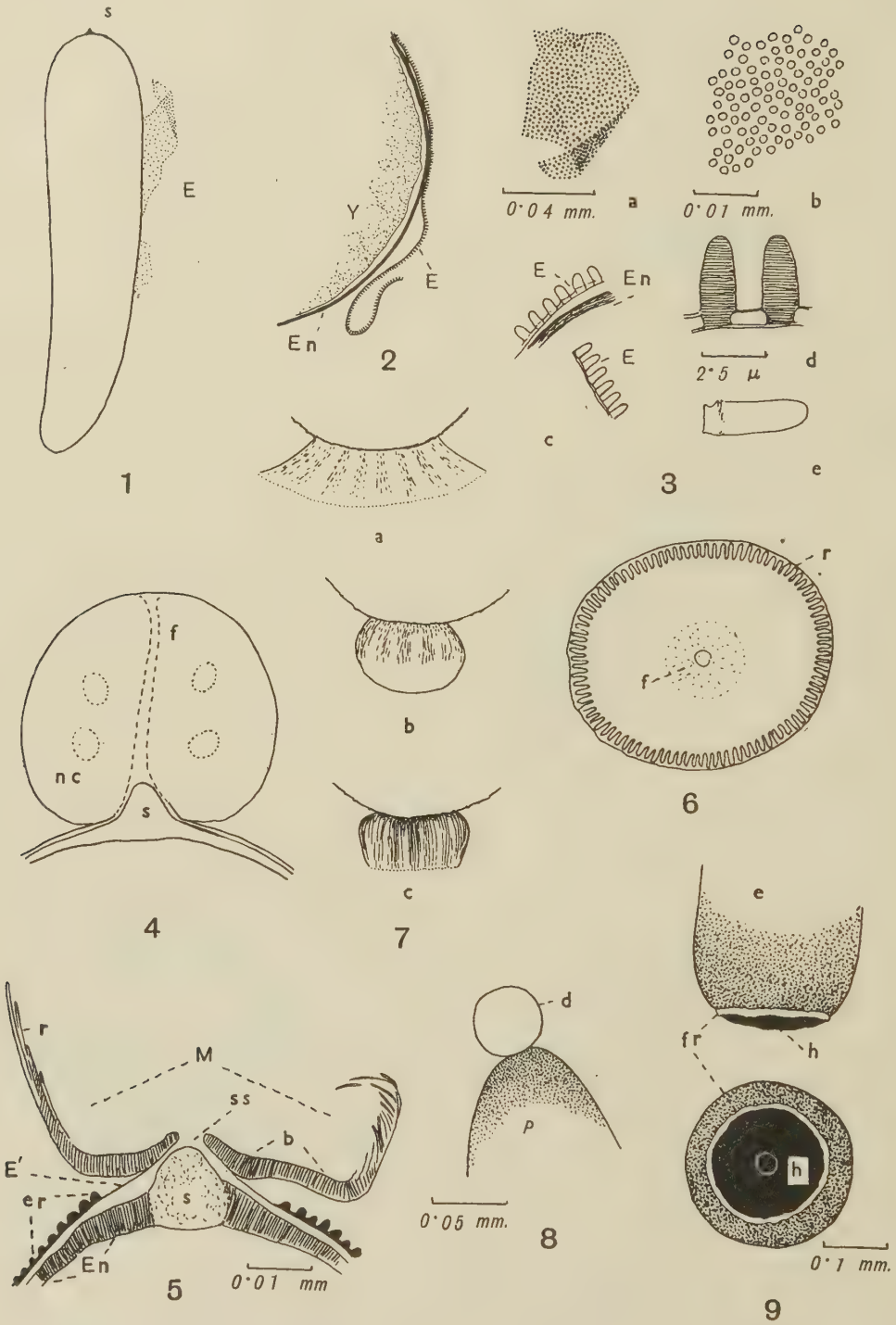
Structural details of the egg of *Culex pipiens*.



Structural details of the egg of *Culex pipiens* and *Theobaldia annulata* (fig. 9).

NOTE.—During discussion of this paper a speaker noted that when *Culex* egg-rafts had been allowed to dry somewhat on filter paper and replaced on water they no longer floated in the normal manner but did so upside down. The most likely explanation is that by drying on filter paper the very readily detached micropilar cups had become detached and left behind. The most hydrophilic portion of the eggs might then quite well have been the posterior polar area which may have then taken on somewhat of the function of the micropilar cups.

REFERENCES.

MARSHALL, J. F., 1944, *The morphology and biology of Culex molestus : observational notes for investigators.* No. 34 (1944), British Mosquito Control Institute, Hayling Island, Hants.

VISHNA NATH, 1924, The egg-follicle of *Culex*. *Quart. J. micr. Sci.* **69** : 151–75.

EXPLANATION OF PLATES.

PLATE 1.

Egg-raft of *C. fatigans*.

FIG. 1. Lateral view of egg-raft showing concave upper surface with apical droplets and convex lower surface with micropilar cups.

2. Egg-raft viewed from above showing arrangement of eggs, each with an apical droplet.

3. Egg-raft viewed from below. In the right lower-hand corner some eggs are seen from which the micropilar cups have become detached in handling.

PLATE 2.

FIG. 1. Showing main structural features of egg. Lateral view. Outline drawn by camera lucida.

2. Micropilar cup in usual condition seen.

3. Micropilar cup as frequently seen when detached and lying expanded on slide. In the lower right-hand quadrant the position occupied by the plaques of the anterior polar specialised area have been indicated as may be seen in a detached egg-cap (*C. pipiens*).

4. Anterior polar specialised area (*C. pipiens*).

5. Posterior polar specialised area showing central area free from projections and the spiked projections around this merging into the normal projections of the exochorion (*C. pipiens*).

PLATE 3.

FIG. 1. Egg showing appearance given by portions of detached exochorion.

2. Section showing exochorion and endochorion, the former in part detached and showing characteristic appearance.

3. a. A portion of exochorion under moderate magnification, showing the regular arrangement of closely set exochorionic projections.
   b. The same very highly magnified.
   c. Section very highly magnified, showing nature of the projections.
   d. Schematic representation of two projections set in the thin double-contoured basal membrane.
   e. Camera lucida drawing of a detached projection.

4. Schematic section through anterior pole of egg whilst still unlaid. Adapted from Vishna Nath.

Fig. 5. Section through anterior pole and micropilar cup of a new-laid egg.
6. Transverse section through micropilar cup.
7. a. Showing condition of micropilar cup when egg in contact with water. Camera lucida drawing.
b. The same immediately after being dragged away from the surface film. Camera lucida drawing.
c. The same some time later when fluid has somewhat evaporated. The usual condition in which the cup is seen.
8. Posterior pole of egg with droplet perched on end. Camera lucida drawing.
9. Anterior pole of egg of *Theobaldia annulata*. a. Lateral view. b. Anterior view.

Plate 4.

Fig. 1. Two eggs of *C. pipiens* after hatching, showing egg-cap. The right-hand figure is a lateral view with the dorsal surface to the right.
2. Schematic drawing showing arrangement of eggs with their dorsal (concave) aspects facing outwards from the middle longitudinal line, the apical droplets at their posterior poles and the expanded micropilar cups in contact with the surface film. The laterally placed eye-spots of the contained larvae are indicated and a larva emerging with egg-cap everted.
3. Egg-raft of *C. fatigans* viewed from above.
4. Egg-raft of *C. molestus* laid autogenously, *i.e.* without the female having had a blood meal. Oval type, 67 eggs.
5. Egg-raft of *C. molestus* from a female that has been given a blood meal. Rectangular type, 86 eggs.
6. Outline of egg-raft of *C. pipiens*, 386 eggs.

Explanation of Lettering.

A Anterior pole of egg.
a Anterior polar specialised area.
b Base of micropilar cup.
bi Inner portion of base of cup covering egg-spike.
bo Outer thickened portion of base of cup.
D Dorsal surface of egg.
d Camera lucida drawing of droplet at posterior pole.
E Exochorion.
E′ Delicate membranous exochorion linking this with base of micropilar cup.
e Small cylindrico-conical projections of exochorion.
er Radially arranged plaques of anterior polar specialised area.
es Enlarged and spiked projections of endochorion.
En Endochorion.
f Micropilar funnel.
f′ Remnant of micropilar funnel and nurse cells sometimes seen as a gelatinous mass.
fr Frill surrounding papilla-free area at anterior pole of egg of *Theobaldia annulata*.
h Hydrophilic area at anterior pole of egg of *Theobaldia annulata*.
M Micropilar cup.
m Area at posterior pole of egg free from exochorionic projections.
nc Nuclei of nurse cells.
P Posterior pole of egg.
p Posterior polar specialised area.
r Wall of micropilar cup.
s Egg-spike.
ss Space into which egg-spike projects. The dotted line indicates the position of reflexion of exochorion on to the micropilar cup.
V Ventral surface of egg.
Y Yolk.

0·2 mm.

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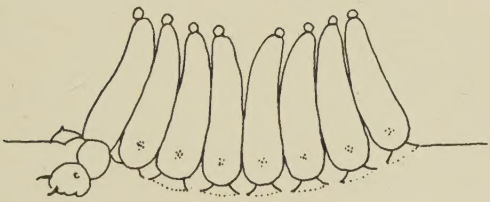

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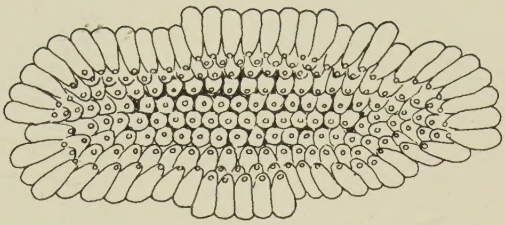

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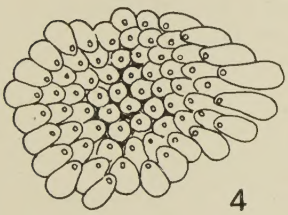

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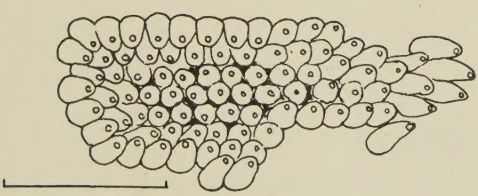

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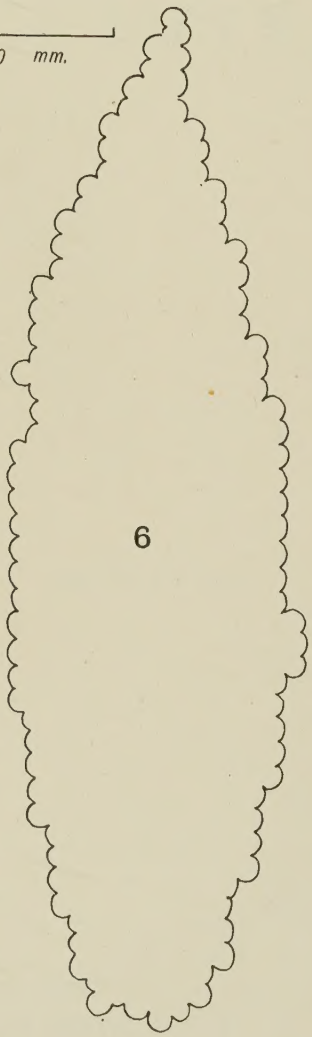

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Details of the egg and egg-rafts in *Culex*.